SUPPORT FOR THE AMENDMENTS

Claim 1 has been amended to correct an obvious mistake.

In claim 1, there are two errors in the positions "132-142" and "116-127" which refer to the positions of the human CK2 α' and β subunit transcripts target region, which includes the 17 to 21 ribonucleotide sequence of the siRNA as specified in claim 1.

This error was corrected based upon:

- Table II page 10 of the specification, which discloses in the second line, a 21 nucleotide target sequence starting at position 132 from the ATG of the human CK2 α' subunit transcript. This target region corresponds to positions 132-152 from the ATG of the human CK2 α' subunit transcript.

- Table III page 11 of the specification, which discloses two overlapping sequences. The second line discloses a 19 nucleotide target sequence starting at position 116 from the ATG of the human CK2 β subunit transcript. The sixth line discloses a 21 nucleotide target sequence starting at position 117 from the ATG of the human CK2 β subunit transcript. These overlapping sequences (positions 116-134 and 117-137) target positions 116-137 from the ATG of the human CK2 β subunit transcript.

Therefore, positions "132-142" and "116-127" were replaced with, respectively, positions "132-152" and "116-137" in claim 1.

No new matter is believed to have been added to the present application by the amendments submitted above.

REMARKS

Claims 1-15, 17-21, 24, 25 and 27 are pending. Favorable reconsideration is respectfully requested.

The present invention relates to a double-stranded oligonucleotide comprising two strands of 19 to 23 nucleotides, each strand consisting, from 5' to 3', of a sequence of 17 to 21 ribonucleotides and two deoxyribo- or ribonucleotides, the 17 to 21 ribonucleotide RNA sequences of said strands being complementary and the two nucleotides of the 3' ends being protruding, wherein the RNA sequence of the sense strand or positive strand is that of a fragment of a transcript of an α, α' or β subunit of a CK2 protein kinase, selected from the group consisting of a), b), c) and d) set forth in Claim 1.

The rejection of the claims under 35 U.S.C. §103(a) over Wyatt in view of Bass and Fosnaugh et al. is respectfully traversed. The cited references fail to suggest the claimed oligonucleotide.

Background of the Invention (see also pages 1-2 of the specification)

The CK2 protein is a pleiotropic serine/threonine kinase composed of two catalytic subunits, α and α' and two identical regulatory subunits, β, associated in the form of heterotetramers.

This protein plays an essential role in the control of many physiopathological processes including cell proliferation, survival and differentiation.

In 2001, CK2 was described as an oncogene and a new pharmacological target for the development of new anticancer drugs (Landesan *et al.*, Oncogene, 2001, 20, 3247-3257)

However, the knock-out of the CK2β gene in mice was shown to be lethal (Buchou *et al.*, Mol. Cell. Biol., 2003, 23, 908-915) and the knock-out of the CK2α' gene in mice was shown to produce infertile mice (Landesan *et al.*, Oncogene, 2001, 20, 3247-3257; Xu *et al.*, Nat. Genet., 1999, 23, 118-121).

Moreover, the few molecules capable of inhibiting CK2 that have been described have the drawbacks of being either not specific or not very active. For example, kinase substrate analogs (ATP analogs), like TBB inhibit the activity of other proteins which use cellular ATP as well as CK2.

Therefore, there is a need for new CK2 inhibitors which can be used to perform functional analysis of each of the CK2 subunits and to screen for new molecules capable of modulating specifically the activity of the CK2 protein. These could be useful as anticancer or antiviral agents (as specified page 1, lines 32-34 and page 2, lines 37-39 of the present application).

The Claimed Invention

Claim 1 is directed to a set of selected siRNAs which specifically and efficiently inhibit one of the three CK2 subunits and target positions 18-74, 259-279, 565-585, 644-664, 720-750, 808-831 and 863-885 (from the ATG start codon) of the human CK2 α subunit transcript, positions 49-69, 132-152, 306-326, 367-387, 427-447, 451-471, 595-615, 735-755, 827-847, 868-888, 949-969 and 988-1008 (from the ATG start codon) of the human CK2 α' subunit transcript, positions 80-100, 116-137, 164-208, 369-389, 400-420, 527-591 and 613-643 (from the ATG start codon) of the human CK2 β subunit transcript.

These siRNAs are not obvious to a person having ordinary skill in the art, for the following reasons.

Additional Data Demonstrating the Advantages of the Claimed siRNAs

The claimed siRNAs which are specific to one of the CK2 subunits represent specific tools and can be used to functionally analyse each of the CK2 subunits as well as to screen for new molecules capable of modulating specifically the activity of the CK2 protein, and in particular anticancer and antiviral agents.

The claimed siRNAs not only, inhibit the expression of the corresponding CK2 subunit *in vitro* (as demonstrated in the examples of the present application) but also produce an effect (phenotype) which is associated with the inhibition of the activity of the corresponding CK2 subunit.

For example, the inventors have shown that the claimed siRNAs targeting the CK2 β subunit disrupt the epithelial phenotype of mammary and intestinal epithelial cells (*Annex I: Deshiere et al., Mol. Cell. Biochem., 2008, 316, 107-113; Annexes II to IV*) thus revealing a new role of CK2 in the maintenance of mammalian cell morphology and cell polarity, which is different from its known role in cell proliferation and survival.

The inventors have shown that the claimed siRNAs have an exquisite specificity compared to other known CK2 kinase inhibitors. As opposed to the CK2 kinase inhibitor, TBB (Figure 3A of *Annex I*), the claimed siRNA targeting the CK2 β subunit has no effect on the cell proliferation rate of mammary epithelial cells (*Annex IV*). TBB triggers a strong apoptotic cell death (Figure 3B of *Annex I*), whereas siRNA-CK2β disrupts the epithelial phenotype, resembling an epithelial to mesenchymal transition (*Annex III*).

The inventors have verified that the phenotype observed in the knock-down cells was due to the silencing of the corresponding CK2 subunit and not to the silencing of a non-specific target (off-target gene silencing effect) by reversing the phenotype (to an epithelial phenotype) after introduction of a chicken mRNA encoding the corresponding CK2 subunit

(mRNA insensitive to degradation by the claimed siRNA targeting the human CK2 subunit mRNA) into the knock-down cells.

In addition, the inventors have shown that the claimed siRNA-CK2β has an antitumoral activity *in vivo* in a mouse model of human tumors (glioblastoma; *Annex V*).

Non-obviousness of the Claimed Invention

The rejection of the claims under 35 U.S.C. §103(a) over Wyatt *et al.* (US 6,440,738) in view of Bass (Nature, 2001) and in view of Fosnaugh *et al.* (US 2003/014732) and over John *et al.* (US 2004/0023855) in view of Fosnaugh *et al.* is respectfully traversed as the cited references fail to suggest the claimed oligonucleotide.

The claimed siRNAs are not obvious to a person having ordinary skill in the art, for the following reasons.

The Examiner considers that Bass teaches that RNA interference is a routinely used gene silencing technique that has proven to be more robust than antisense technique by working more often, but this is not the case.

Bass discloses the work of Elbashir *et al.* (Nature, 2001, 411, 494-498), which is the first report of siRNA inhibition in mammalian cells. Therefore, Bass does not disclose routine use of siRNA in mammalian cells.

Furthermore, it is worth noting that the Patent Application US 2003/0143732 by Fosnaugh et al., was filed on August 28, 2001, *i.e.* at the time siRNA inhibition in mammalian cells was first reported by Elbashir *et al.*, Nature, 2001, 411, 494-498, as highlighted in Bass et al., Nature, 2001, 411, 428-429.

The Examiner considers that Fosnaugh *et al.* comprises a detailed blueprint for how to make and use inhibitory siRNAs to target any known gene, but it is not the case.

Fosnaugh *et al.*, only discloses a series of vague and theoritical considerations to make siRNAs.

It is worth noting that in paragraph [0082] Fosnaugh *et al.*, disclose the generation and the assay of a library of $\underline{4^{19}}$ siRNAs which is totally unrealistic.

Fosnaugh *et al.*, make statements about various parameters which can be used to determine which sites are the most suitable target sites within the RNA target sequence which are so vague that the person having ordinary skill in the art would not know how to use these parameters (paragraphs [083],[0232]).

Fosnaugh *et al.* is based mainly on the blind screening of thousands of siRNAs representing all the sequences having sequence complementarity with all fragments of a particular length (for example 23 nucleotide fragments) contained within the target sequence.

A non-limiting example of library is presented in Table I page 32. Table I shows a library of 161 siRNAs targeting 19 nucleotide targets overlapping by 1 nucleotide and starting at position 3 of the mRNA transcript of interest. The complete screening of the 19 nucleotide targets according to the method of Fosnaugh *et al.*, involves the generation of 17 other libraries starting at any position between position 3 and position 21 (positions 4, 5,...., 18, 19 and 20). Even if the number of targets was limited using standard parameters like GC content, self-folding, absence of GGG and CCC, this method would still represent excessive work, which goes beyond the normal experimental and working capacity of the person ajudged to have ordinary skill in the art.

A mere catalogue of possibilities to make siRNAs, which are either described in vague terms or not practicable because of excess work and are not illustrated by any experimental data showing that inhibitory siRNAs to the target gene were made, cannot be considered as a technical teaching to make and use inhibitory siRNAs to target any known gene.

Furthermore, Fosnaugh *et al.* is totally silent about the specificity of the siRNAs. Since the siRNAs of Fosnaugh *et al.* are generated blindly they will include non-specific siRNAs targeting non-specific RNA targets.

Therefore, Fosnaugh *et al.*does not teach how to make specific siRNAs.

The Examiner considers that the claimed siRNAs are obvious in light of Wyatt *et al.* that teach one antisense oligonucleotide in Table 1, the sequence of which, SEQ ID NO: 60 is targeted to a region sharing 16 nucleotides with the elected target region represented by SEQ ID NO: 26. However, it is only with the foreknowledge of the invention that the Examiner is making this assertion.

A person having ordinary skill in the art would not have had any motivation to make siRNA sequences targeting the regions disclosed in the Table 1 of Wyatt *et al.*, since it is well known in the art that antisense oligonucleotides and siRNAs don't have the same targets. Even, if the skilled man had such motivation, he would have chosen the more active targets, which like the targets of the claimed siRNAs, show at least 80 % inhibition of human CK2-beta expression. In this case, he would have had selected the sequences SEQ ID NO: 22, 49, 51, 53, 55, 56, 58, 69, 73, 76, 82, 83, 84 and 91 which target the following positions of the CK2-beta mRNA transcript starting from the ATG initiation codon: 1282-1301, (5'UTR), 8-27, 23-42, 33-52, 40-59, 69-88, 238-257, 334-353, 371-390, 482-501, 492-511, 520-539 and 695-714. These sequences which are different from the sequences of the targets of the claimed siRNAs, demonstrate that it is fortuitous that SEQ ID NO: 60 in Wyatt *et al.* overlap with SEQ ID NO: 26 in the present invention.

Therefore, the claimed siRNAs are not obvious in light of the antisense oligonucleotides in Table 1 of Wyatt *et al.*.

For these reasons, contrary to the Examiner's opinion the person having ordinary skill in the art could not arrive at the claimed invention by combining the teaching of Wyatt *et al.* to make and test a multitude of oligonucleotides targeted throughout the gene sequence, combined with the teaching of Fosnaugh *et al.*.

Making and testing a multitude of oligonucleotides targeted throughout the gene sequence is only a theoretical solution which cannot be tested since it goes beyond the normal working capacity of the person having ordinary skill in the art.

The citation of John *et al.*, is based on the incorrect assertion that siRNAs can be based on any portion of a messenger RNA molecule.

This is not correct, as not all siRNAs work: only a small portion of siRNAs selected for targeting a region of a gene show efficient gene silencing properties. The instability of the siRNA *in vivo*, its inability to interact with components of the RNAi machinery, the inaccessibility of the target mRNA due to local secondary structural constraints, the interaction of the target mRNA with regulatory proteins or the instability of the target mRNA are possible causes for the failure of most tested siRNAs.

Li *et al.*, 2007 (Annex 5) which was published after the filing of the present Application was provided to demonstrate that four years after the filing of the present Application, even though many prediction methods have been developed, the making of siRNAs with high efficiency and high specificity to a target still poses significant problems.

The Examiner refers to page 87, column 5 of Annex 5 to demonstrate that efficient siRNAs could be made with a high expectation of success. However, these results were obtained using the method developed by Reynolds *et al.* in 2004. This method was not available at the date of filing of the present application. Furthermore, almost all, if not all the methods cited in Annex 5 which were published after 2004 and so were not available at the date of filing of the present application.

This demonstrates that in 2003, there was no reliable target-selection method to design siRNAs. Nevertheless, having a reliable target-selection method to design is just a first step to make functional siRNAs. It does not ensure that each siRNA will work for the reasons already mentioned above.

The inventors were the first to make siRNAs targeting the human CK2 protein. Therefore, they could not use the teaching from others to select the most suitable target site in the CK2 subunits mRNA sequences.

High-throughput screening as disclosed by Fosnaugh *et al.*, could not be used for the CK2 protein, due to the lack of suitable CK2 antibodies (Laramas *et al.*, European Journal of Cancer, 2007, 43, 928-934; *Annex VI*) and the fact that phenotypic observation was the only reliable method of monitoring the effects of a putative siRNA on a target cell/tissue.

Therefore, the identification of siRNAs with high efficiency and high specificity to its human CK2 subunit target would not have been considered routine work for one of ordinary skill in the art at the time the invention was made.

Therefore, a person having ordinary skill in the art would not have arrived at the claimed invention, because he would have expected to have had to perform undue experimentation.

In view of the foregoing, the claimed siRNAs are not suggested by the cited references. It is only with the foreknowledge of the invention and the knowledge of siRNA technology at the present time, that the Examiner could make the assertion that the generation of siRNA material was obvious.

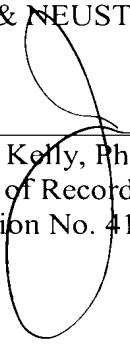
This conclusion relies upon an *ex post facto* analysis, starting from the invention and showing how it might be easily arrived at starting from Wyatt *et al.* in view of Bass and in view of Fosnaugh *et al.* or starting from John *et al.* in view of Fosnaugh *et al.*.

In view of the foregoing, withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



James J. Kelly, Ph.D.
Attorney of Record
Registration No. 41,504

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 08/07)